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A Light and Scanning Electron Microscopic Study of the Closing Apparatus in Tintinnid Ciliates (Ciliophora, Spirotricha, Tintinnina): A Forgotten Synapomorphy

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Abstract

A membranous closing apparatus shuts the lorica opening in disturbed tintinnids of six genera belonging to four families. The homology of the apparatuses is investigated, using data from the literature and Mediterranean tintinnids studied in vivo and by scanning electron microscopy. Morphological and functional similarities indicate that the foldable closing apparatus is not only a synapomorphy of the genera *Codonella* (Codonellidae) and *Dictyocysta* (Dictyocystidae), as suggested 80 years ago, but also of *Codonaria* (Codonellidae) and *Codonellopsis* (Codonellopsidae). In *Codonaria*, *Codonella*, and *Dictyocysta*, the apparatuses merge posteriorly into membranous lorica sacs, which probably represent homologous structures. The diagnoses of these genera are improved according to the new findings. The close relationship of *Codonella*, *Codonellopsis*, and *Dictyocysta* is also inferred from small subunit rRNA phylogenies and the ultrastructure of the capsules. It contradicts the current lorica-based classification of the tintinnids. The assumption that the diaphragm-like apparatus in the genera *Salpingacantha* and *Salpingella* is not homologous to the foldable ones in the genera mentioned above is supported by molecular and cytological features.

Keywords

Classification; lorica sac; marine protists; morphology; phylogeny; plankton; taxonomy; ultrastructure

The closing apparatus, first observed in the tintinnid *Codonella aspera* by Fol (1883; misidentified as *Codonella galea*), is a membranous structure that shuts the lorica opening when the ciliates are disturbed. This structure occurs in tintinnids of six genera belonging to four families: *Codonaria* and *Codonella* of the family Codonellidae; *Codonellopsis* of the family Codonellopsidae; *Dictyocysta* of the family Dictyocystidae; as well as *Salpingacantha* and *Salpingella* of the family Tintinnidae (Table 1). In the present paper, the homology of the closing apparatuses, as assumed by Hofker (1931) for the genera *Codonella* and *Dictyocysta*, is investigated. To this end, the motility and ultrastructure of the apparatuses in *Codonella aspera*, *Dictyocysta lepida*, and *Dictyocysta mitra* are examined and compared with data from the literature.

MATERIALS AND METHODS

Sampling

Various tintinnid species (Table 2) were collected with a 10- μ m plankton net from surface waters of the Mediterranean Sea near the village of Villefranche-sur-mer (France; 43°41'10"N, 07°18'50"E) in May 2008, October 2008, and October 2009. The water temperatures were 17 °C, 18 °C, and 20 °C, respectively, and the salinity ranged from 37 to 38 PSU.

Morphological studies

Live specimens from fresh samples were studied at 1,000 $\times$ magnification by means of a compound microscope equipped with a high-power oil immersion objective as well as bright-field and interference contrast optics. For scanning electron microscopy, specimens were fixed for 30 min in a modified Parducz' solution made of six parts of 2% (w/v) OsO₄ in artificial sea water and one part of saturated aqueous HgCl₂ (Valbonesi and Luporini 1990); further steps followed Foissner (1991). The classification of the tintinnid ciliates is according to Lynn (2008).

Terminology

The term "closing apparatus" was probably introduced by Kofoed and Campbell (1929) and represents the translation of the German term "Verschlußapparat" used by Entz (1884). "Aparato de cierre" (Balech 1959), "opercule" (Laval-Peuto 1994), "Schließapparat" (Biedermann 1893), and "Schließklappen" (Entz 1909) are further synonyms. The latter term was also used by Entz (1909) for the agglomeration of particles at the lorica opening in *Stenosemella* species as described, for instance, by Agatha and Tsai (2008). The term "lorica sac" is introduced for the membranous envelop that lines the lorica bowl in some genera and merges anteriorly into the closing apparatus. This term should replace the synonyms "Hüllhaut", "Hüllmembran", "Schutzmembran" (Biedermann 1893), "Sack" (Hofker 1931), and "sac interne" (Laval-Peuto 1994).

RESULTS

In *Codonella* and *Dictyocysta*, closing apparatuses occurred in all loricae that were inhabited and ~ 18% ($n=17$) of the empty ones studied in the scanning electron microscope; in contrast, these structures were not observed in *Codonellopsis schabi*, *Salpingella acuminata*, and the other co-occurring tintinnids (Table 2). Because *Codonella aspera* was more abundant than *Dictyocysta lepida* (Fig. 23) and *D. mitra* (Fig. 19-22), the observations are more detailed in that species (Table 3 and Fig. 1-18, 28).

Motility

The loricae of the *Codonella* and *Dictyocysta* species consist of a collar and a bowl separated by a constriction, at which the proximal end of the membranous closing apparatus seemed to originate in the live tintinnids (Fig. 1, 4, 5, 16, 19, 23). Swimming ciliates, with spread collar membranelles and moving somatic cilia, partially emerged through the open closing apparatuses, whose cylindroidal membranes loosely lined the lorica collars. When disturbed, the ciliates rapidly (<1 s) retracted into the lorica bowls by a contraction of their stalks. Simultaneously, the closing apparatuses performed a complex folding and the lorica openings were quickly closed (<1 s; Fig. 1, 2, 4-6, 23). In these contracted tintinnids, the collar membranelles were arranged like an iris diaphragm and vibrated. However, there was no indication that the ciliates participated in the closing processes (e.g. by means of the cilia). When the ciliates commenced to emerge from the lorica, the closing apparatuses slightly opened and shut according to the forward and backward movements of the cell.

Only one out of 15 ciliates investigated under the compound microscope restarted swimming; the majority contracted maximally and eventually burst, while the closing apparatuses remained shut. The loricae were never abandoned.

Ultrastructure

In preserved specimens, it is obvious that the foldable closing apparatuses merge posteriorly into lorica sacs, which separated from the inner wall of the lorica bowls probably due to a shrinkage caused by the preservatives (Fig. 9-12, 18-22, 28). The membranes of the apparatuses are $\sim 0.1\text{--}0.2\text{ }\mu\text{m}$ thick and possibly composed of two layers (Table 3 and Fig. 8, 9, 13-15, 17, 18). Occasionally, contracted cells with widely open apparatuses are found in the scanning electron microscope, allowing a view inside the lorica sacs. The cylindroidal membranes of the open apparatuses reveal longitudinal furrows and ribs, while the lorica sacs are smooth or only slightly wrinkled (Fig. 7, 8, 10, 12, 14, 15, 17-22). In *Codonella aspera*, the usually 10 longitudinally and equidistantly arranged shallow furrows on the inner wall of the open closing apparatus correspond to ribs $\sim 0.3\text{ }\mu\text{m}$ high on the outer wall (Table 3 and Fig. 3, 7, 8, 14, 17). Between two furrows each, six to eight longitudinal ribs $\sim 0.1\text{--}0.2\text{ }\mu\text{m}$ thick and $\sim 1.2\text{--}1.8\text{ }\mu\text{m}$ apart extend on the inner wall of the apparatus but do not reach the anterior margin; occasionally, they are proximally bifurcated and faintly visible on the outer wall (Fig. 15). The ribs on the outer wall form the tops of the usually 10 conspicuous folds in the shut closing apparatus (Fig. 1, 2, 4-6, 9-13, 15, 18). The $\sim 0.2\text{--}0.4\text{ }\mu\text{m}$ thick folds are radially arranged in the lorica opening, longitudinally orientated, and triangular: they are $\sim 20\text{ }\mu\text{m}$ long and increase to an average height of $5.7\text{ }\mu\text{m}$ at the anterior margin. Slightly inclined or vaulted triangular valves separate the folds. A ring-shaped crease near the proximal ends of the triangular folds marks the base of the closing apparatus, viz., the site which is probably attached to the lorica constriction in live specimens (Fig. 10, 12, 22). This structure is often bright in the scanning electron micrographs and seems to be rather stiff, but it is apparently not connected to the ribs of the closing apparatus, as the latter is occasionally sagged in preserved specimens (Fig. 22). Scanning electron micrographs reveal a minute opening $\sim 1\text{--}2\text{ }\mu\text{m}$ across in the centre of all shut closing apparatuses (Fig. 2, 11-13), through which cytoplasm flows out of the sac in burst specimens. There are no apparent contacts between the cell and the closing apparatus that might indicate an active closing mechanism. The globular swellings at the ends of the collar membranelles (Fig. 7-10, 15, 17-22) probably result from a suboptimal preservation, as they were not recognizable in the live specimens.

The motility and general morphology of the closing apparatuses in *Dictyocysta lepida* and *D. mitra* are identical to those in *Codonella aspera*. The closing apparatus of *D. lepida*, studied in vivo, possesses eight folds ($n=4$; Fig. 23). In *D. mitra*, the number of folds could not be elucidated, as the specimens were only found with open apparatuses in the scanning electron microscope (Fig. 19-22).

DISCUSSION

Formation

The formation of the closing apparatus and the lorica sac probably takes place after lorica construction (Hofker 1931) and may require certain environmental conditions and/or a particular stage in the tintinnid's life cycle. Hence, not all specimens of a species possess these structures (Daday 1887; Entz 1909; Hofker 1931). According to Hofker (1931) and Kofoid and Campbell (1939), the closing apparatus and lorica sac are discarded when the cell abandons the lorica. In *Codonella* and *Dictyocysta* collected in Villefranche-sur-mer, closing apparatuses occurred in all inhabited loricae, while they were absent in $\sim 82\%$ of the empty ones, probably because they were not formed or lost during the handling; the

alternative, that the ciliates left the loricae and took the apparatuses and sacs along, seems unlikely.

Entz (1909) assumed that the lorica sac represents the shedding product of the cell surface, possibly the old perilemma, which is subsequently incorporated into the lorica wall. The present results reject this hypothesis, as the sac neither shows hollow bulges caused by the “skinned” cilia nor matches the lorica matrix in its chemical composition (Hofker 1931). Furthermore, the sac separates from the lorica bowl after application of preservatives (this study; Hofker 1931). So, the lorica sac seems to be a distinct structure that forms in a similar way to the lorica matrix (i.e. through secretion/exocytosis of material by the cell).

Morphology and motility

The findings of the present light and scanning electron microscopic study agree with the literature data on the closing apparatuses in *Codonaria*, *Codonella*, *Codonellopsis*, and *Dictyocysta* (Fig. 24-27, 29-38; Biedermann 1893; Brandt 1906, 1907; Daday 1887; Entz 1884, 1885, 1909; Fol 1883; Hofker 1931; Laval-Peuto 1994). In these genera, the apparatuses form 8–24, usually 12 conspicuous folds and merge posteriorly into lorica sacs (Table 3; Biedermann 1893; Entz 1884; Hofker 1931; Kofoid and Campbell 1939), except for the genus *Codonellopsis* in which lorica sacs were not mentioned (Brandt 1906; Kofoid and Campbell 1939).

In the following, different closing mechanisms are discussed. (i) Automatic closure. Black iron haematoxylin stains indicate elastic fibres in the ring-shaped creases at the bases of the closing apparatuses (Entz 1909); however, the creases are invariably attached to the lorica constrictions in live specimens and thus do not show any contractility or elasticity (this study; Biedermann 1893; Hofker 1931). In addition, the contracted specimens with widely open apparatuses studied in vivo during oxygen shortage (Hofker 1931) and in the scanning electron microscope (Fig. 7, 14, 17, 20) suggest that the apparatuses are directly or indirectly shut by the ciliates and that their creases, ribs, and furrows are probably only responsible for an ordered folding (Biedermann 1893). (ii) Active closure by the ciliate. Two projections of the peristomial rim attached to the lorica wall cause the collapse of the lorica in contracting specimens of the tintinnid *Nolaculus hudsonicus* (Snieszek et al. 1991). In the taxa under consideration, however, contacts between the cell and the closing apparatus were neither observed (Fig. 7-10, 14, 17, 18, 20) nor mentioned in the literature. Furthermore, the tintinnids did not display special ciliary movements during the rapid closure of the apparatuses. (iii) Indirect closure by the contracting ciliate. In the loricate peritrich *Thuricola*, a half-cylindrical membrane extends longitudinally between a closing valve and the lorica bottom. The contracting ciliate closes the lorica with the valve through a deformation of the membrane (Gruber 1879; Kahl 1935). A similar process is unlikely in the tintinnids, as the lorica sac is attached to the lorica wall in live specimens and hence cannot be deformed. Hydrodynamic processes are suggested for the collapse of the bilateral lorica in the tintinnid *Nolaculus bicornis*, i.e. water expelled from the lorica by the contracting ciliate generates a low pressure (Snyder and Brownlee 1991). The retracted ciliates with widely open closing apparatuses seem to rule out a hydrodynamic closure, but the contraction of these specimens might have been too slow for the generation of a low pressure. Indeed, the swollen tips of their cilia indicate a suboptimal, possibly delayed preservation.

Once folded, the closing apparatuses remain shut until the pressure of the extending tintinnids forces apart the valves of the apparatuses, i.e. they even do not open in unpreserved empty loricae (this study; Biedermann 1893; Hofker 1931); perhaps, adhesion between the membranous fold layers prevents an automatic opening.

In *Salpingacantha* and *Salpingella*, the closing apparatuses were poorly described and data on their motility are not available. According to Balech (1959) and Kofoed and Campbell (1929, 1939), the apparatuses are diaphragm-like and apparently lack folds and lorica sacs (Fig. 39-47).

Function

Biedermann (1893) and Daday (1887) assumed a protective function of the closing apparatuses, as they quickly shut in disturbed tintinnids. In contrast to cysts, however, the folded apparatuses apparently do not hermetically shut, but leave a minute opening in their centres (Fig. 2, 11-13; this study; Kofoed and Campbell 1939). Accordingly, they represent an ineffective protection against harmful environmental conditions and digestion by predators, such as copepods. On the other hand, the foldable apparatuses might shield the ciliates from predatory and ectoparasitic dinoflagellates, which are lethal to many other tintinnid genera (Agatha and Riedel-Lorjé 2006; Uchida et al. 1997). Furthermore, the closing apparatuses might reduce the dispersal of infochemicals released by the tintinnids (e.g. dimethyl sulphide from the digestion of phytoplankton; Belviso et al. 1990; Christaki et al. 1996; Steinke et al. 2002), decreasing the probability that the ciliates are detected by approaching predators (Pohnert et al. 2007; Steinke et al. 2006). Especially in tintinnids with windows in the lorica bowl, such as *Dictyocysta mitra* (Fig. 19-22), the latter function necessitates the presence of lorica sacs, indicating that they evolved together with the closing apparatuses.

Taxonomic and phylogenetic aspects

In this section, I pragmatically follow Kofoed and Campbell (1929, 1939) in their species synonymizations, but provide the original identifications in brackets.

Several congeners of *Codonella aspera* (Fig. 1-18, 24-28; this study; Balech 1959; Daday 1887: *C. lagenula*; Fol 1883: *C. galea*; Kofoed and Campbell 1939) possess a foldable closing apparatus and often show a lorica sac: the type species *C. galea* (Balech 1959; Brandt 1906; Campbell 1942; Hofker 1931), *C. amphorella* (Biedermann 1893; Brandt 1906; Campbell 1942), *C. apicata* (Brandt 1906: *C. perforata*; Campbell 1942; Entz 1909: *C. perforata*; Kofoed and Campbell 1939), *C. acuta*, *C. cuspidata*, *C. pacifica*, *C. tropica* (Kofoed and Campbell 1939), *C. elongata* (Biedermann 1893: *C. polymorpha*; Entz 1909: *C. galea*), *C. poculum* (Campbell 1942), and *C. nationalis* (Brandt 1906; Hofker 1931). Furthermore, foldable closing apparatuses and lorica sacs occur in *Codonaria* species: the type species *C. cistellula* (Fig. 29-32; Campbell 1942; Entz 1908, 1909; Hofker 1931; Kofoed and Campbell 1939), *C. australis* (Brandt 1906: *C. cistellula*; Kofoed and Campbell 1939), *C. lata*, and *C. mucronata* (Kofoed and Campbell 1939).

The occurrence of foldable closing apparatuses and lorica sacs is not restricted to the family Codonellidae, but these structures are also found in the genus *Dictyocysta* of the family Dictyocystidae, viz., in the type species *D. elegans* (Biedermann 1893), *D. lepida* (Fig. 23, 33-35; this study; Entz 1885: *D. templum*; Hofker 1931), *D. mitra* (Fig. 19-22, 36, 37; this study; Biedermann 1893: *D. elegans*; Entz 1909), *D. duplex* (Brandt 1906: *D. templum*), *D. mexicana* (Hofker 1931: *D. lepida*), *D. minor*, *D. mülleri*, *D. occidentalis*, *D. pacifica*, *D. spinosa* (Kofoed and Campbell 1939), *D. nidulus* (Entz 1909: *D. templum*), *D. reticulata* (Daday 1887: *D. templum*; Hofker 1931: *D. lepida*; Kofoed and Campbell 1939), *D. speciosa* (Biedermann 1893: *D. elegans*, *D. templum*), and *Dictyocysta* sp. (Laval-Peuto 1994). Only for *D. polymorpha*, morphometric data and illustrations are apparently lacking (Biedermann 1893; Entz 1884).

The genus *Codonellopsis* of the family Codonellopsidae also includes at least two species with foldable closing apparatuses: *C. minor* (Kofoed and Campbell 1939) and *C. pacifica* (Fig. 38; Brandt 1906: *C. orthoceras*); however, evidence for the presence of a lorica sac is missing.

In contrast to the genera mentioned above, the closing apparatuses in *Salpingella* and *Salpingacantha* (family Tintinnidae) are diaphragm-like; folds, lorica sacs, and closing movements were not mentioned. Several members of the genus *Salpingella* possess such a closing apparatus: the type species *S. acuminata* (Kofoed and Campbell 1939, while not observed in this study), *S. decurtata* (Fig. 39, 40; Balech 1959), *S. curta* (Fig. 44; Balech 1959; Kofoed and Campbell 1939), *S. rotundata* (Fig. 41-43), and *S. subconica* (Kofoed and Campbell 1939). In *Salpingacantha*, diaphragm-like closing apparatuses occur in *S. exilis* (Fig. 47), *S. perca* (Fig. 45; Campbell 1942; Kofoed and Campbell 1929, 1939), and *S. unguiculata* (Fig. 46; Kofoed and Campbell 1929, 1939).

The morphological and functional similarities found in this study and the literature data indicate that the foldable closing apparatus represents a synapomorphy of the genera *Codonaria*, *Codonella*, *Codonellopsis*, and *Dictyocysta*, while the diaphragm-like closing apparatus in *Salpingacantha* and *Salpingella* is probably not homologous (see below). In *Codonaria*, *Codonella*, *Dictyocysta*, and probably *Codonellopsis*, the foldable closing apparatuses merge posteriorly into lorica sacs, which very likely represent homologous structures. The close relationship of the genera *Codonella*, *Codonellopsis*, and *Dictyocysta* is also inferred from sequence analyses of the small subunit ribosomal RNA gene (SSrRNA; Kim et al. 2010; Strüder-Kypke and Lynn 2008). In the BLAST analysis of the SSrRNA gene sequences, *Codonella aspera* (from Villefranche-sur-mer, sequenced by Charles Bachy, Université de Paris-Sud) is very similar to that of *Codonella apicata* (99.59%; GenBank number EU399531), *Dictyocysta reticulata* (98.98%; EU399532), *Codonellopsis americana* (98.58%; AY143571), and *Codonellopsis nipponica* (98.51%; FJ196072). Likewise, the genera *Codonaria*, *Codonella*, *Codonellopsis*, and *Dictyocysta* share the same type of capsules (probably the extrusive organelles of tintinnids; Laval-Peuto and Barria de Cao 1987). Unfortunately, the somatic ciliary pattern is unknown in *Codonella* species with a closing apparatus (see below) as well as in the genera *Codonaria* and *Dictyocysta* (Agatha and Strüder-Kypke 2007). Future cytological studies and cladistic analyses will thus further test the supposed relationships.

The diaphragm-like closing apparatus of *Salpingacantha* and *Salpingella* is considered a convergence to the foldable apparatus in the genera mentioned above because of (i) its different morphology, (ii) a different position of *S. acuminata* (EU399536) in the SSrRNA phylogenies (own data; Strüder-Kypke and Lynn 2008), and (iii) a different orientation of the adoral zone of membranelles in contracted specimens (oblique vs. perpendicular to the main cell axis; own data; Laval-Peuto 1994; Laval-Peuto and Brownlee 1986; Lynn and Small 2002; Small and Lynn 1985). Possibly, the diaphragm-like closing apparatus represents a synapomorphy of the genera *Salpingacantha* and *Salpingella*.

Conclusion

A close relationship between the genera *Codonella* and *Dictyocysta* due to the shared possession of a foldable closing apparatus was assumed by Hofker (1931) and is supported by the present findings, literature data, SSrRNA phylogenies, and cytological characteristics. In the past 80 years, however, the closing apparatus and the lorica sac were forgotten and have not been considered in revisions of the tintinnid classification (Table 1; Campbell 1954; Corliss 1979; Laval-Peuto 1994; Lynn 2008; Lynn and Small 2002; Small and Lynn 1985). Actually, cladistic (Agatha and Strüder-Kypke 2007) and molecular analyses (Strüder-Kypke and Lynn 2003, 2008) contradict the exclusively lorica-based current

classification. Likewise, the rediscovered features, closing apparatus and lorica sac, demonstrate the low significance of the general lorica wall structure for higher taxonomic ranks: *Codonaria* and *Codonella* have agglomerated lorica bowls and collars; in *Codonellopsis* and the majority of *Dictyocysta* species (e.g. *D. lepida*), the loricae consist of agglomerated bowls and hyaline collars; and some *Dictyocysta* species (e.g. *D. mitra*) possess loricae with hyaline collars and bowls. So, it is obvious that a far-reaching revision is required. However, at present, this is impossible, as only species ~ 2% of the ~ 1,200 tintinnid have been studied by SSrRNA gene sequencing or cytological analysis. While the reconstruction of the tintinnid evolution and the establishment of a natural classification thus need a joint effort of morphological and molecular taxonomists, the present findings at least permit an improvement of the genus diagnoses in *Codonaria*, *Codonella*, and *Dictyocysta*.

Codonaria Kofoid & Campbell, 1939

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- 1939 *Codonaria* g. n.—Kofoid and Campbell, *Bull. Mus. Comp. Zool. Harv.*, **84**:55.
- 1968 *Codonaria* Kofoid and Campbell 1939—Loeblich and Tappan, *J. Protozool.*, **15**:186.
- 1979 *Codonaria* Kofoid and Campbell 1939—Corliss, *The Ciliated Protozoa*, 2nd ed.:303.
- 1994 *Codonaria* Kofoid et Campbell, 1939—Laval-Peuto, *Traité de Zoologie*, II (2):207.
- 2001 *Codonaria* Kofoid and Campbell 1939—Aescht, *Denisia*, **1**:44.
- 2008 *Codonaria* Kofoid and Campbell 1939—Lynn, *The Ciliated Protozoa*, 3rd ed.:351.
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Improved diagnosis—Lorica agglomerated, firm, urceolate, divided into a collar and a bowl by a constriction, rarely and sparsely fenestrated. Collar generally obconical, with subapical cone, usually shorter than the globular, ellipsoidal, or obovoidal bowl. With lorica sac and foldable closing apparatus.

Type species—*Cyttarocyliis cistellula* Fol, 1884 (by original designation).

Discussion—In contrast to Fol (1884), several authors observed a closing apparatus in the type species of the genus *Codonaria* (Fig. 29-32; Entz 1908, 1909; Hofker 1931; Kofoid and Campbell 1939). Besides the closing apparatus, Hofker (1931) found a lorica sac; however, Kofoid and Campbell (1939) only included the former feature into the genus description/diagnosis. The fenestration observed in some species is probably caused by the loss of imbedded particles.

Codonella Haeckel, 1873

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- 1873 *Codonella*—Haeckel, *Jen. Z. Med. Naturwiss.*, **7** (year **1871**):564.
- 1907 *Codonella* H.—Brandt, *Ergebn. Plankton-Exped. Humboldt-Stiftung*, **3La**:73.
- 1909 *Condella*—Entz, *Arch. Protistenk.*, **15**:102 (incorrect subsequent spelling).
- 1924 *Codonella* Hck.—Jørgensen, *Mediterranean Tintinnidae*:90.
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- 1929 *Codonella* Haeckel emended Brandt emended Jörgensen—Kofoid and Campbell, *Univ. Calif. Publ. Zool.*, **34**:51 (revision).
- 1939 *Codonella* Haeckel emended—Kofoid and Campbell, *Bull. Mus. Comp. Zool. Harv.*, **84**:42 (revision).
- 1968 *Codonella* Haeckel 1873—Loeblich and Tappan, *J. Protozool.*, **15**:186.
- 1979 *Codonella* Haeckel 1873—Corliss, *The Ciliated Protozoa*, 2nd ed.:303.
- 1987 *Codonnella*—Laval-Peuto and Barria de Cao, *Ile Réunion. Scientif. GRECO 88, Trav. C.R.M.*, **8**:56 (incorrect subsequent spelling).
- 1994 *Codonella* Haeckel 1873—Laval-Peuto, *Traité de Zoologie*, II (2):207.
- 2001 *Codonella* Haeckel 1873—Aescht, *Denisia*, **1**:44.
- 2008 *Codonella* Haeckel 1873—Lynn, *The Ciliated Protozoa*, 3rd ed.:351.

Non-*Codonella* Canu and Bassler, 1928, *Bull. Soc. Sci. Seine-et-Oise*, **8**:25 (junior homonym; bryozoan genus).

Improved diagnosis—Lorica agglomerated, firm, urceolate, divided into a collar and a bowl by a constriction and/or an inner projecting rim, rarely with scattered irregular windows. Collar cylindroidal or obconical, usually shorter than bowl, rarely with a minute hyaline rim. Bowl globular, ellipsoidal, or obovoidal, rarely with a posterior process. With lorica sac and foldable closing apparatus.

Type species—*Codonella galea* Haeckel, 1873 (by subsequent designation by Brandt 1907).

Discussion—In contrast to Haeckel (1873), several authors described and illustrated a lorica sac and closing apparatus in the type species of the genus *Codonella* (Balech 1959; Brandt 1906; Hofker 1931). Like in *Codonaria*, the irregular lorica windows might result from the loss of agglomerated particles.

The generic affiliation of the freshwater species *Codonella cratera* (Leidy, 1877) Imhof, 1885 is probably incorrect, as a closing apparatus and lorica sac were never mentioned (Foissner et al. 1999). Since the cytological features of the type species *Tintinnopsis beroidea* Stein, 1867 are insufficiently known (Agatha and Riedel-Lorjé 2006), a transfer of *C. cratera* to the genus *Tintinnopsis* or the establishment of a new genus for this species are premature. For the present, the generic assignment of *C. cratera* is thus not changed, but its somatic ciliary pattern cladistically analysed by Agatha and Strüder-Kypke (2007) is not any longer representative for the genus.

Dictyocysta Ehrenberg, 1854

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- 1854 *Dictyocysta*—Ehrenberg, *Monatsber. Akad. Wiss. Berl.*, **1854**:71.
- 1854 *Dictyocysta*—Ehrenberg, *Monatsber. Preuss. Akad. Wiss. Berl.*, **1854**:236.
- 1885 *Dictyocista*—Entz, *Mitt. zool. Stn Neapel*, **6**:203 (incorrect subsequent spelling).

- 1915 *Dictyocysta*—Apstein, *Sber. Ges. naturf. Freunde Berl.*, **1915**:123 (compilation of nomina conservanda).
- 1929 *Dictyocysta* Ehrenberg emended—Kofoid and Campbell, *Univ. Calif. Publ. Zool.*, **34**:285 (revision).
- 1929 *Dyctiocysta*—Massuti Alzamora, *Notas Resúm. Inst. esp. Oceanogr.*, ser. II, **32**:11 (incorrect subsequent spelling).
- 1930 *Dystiocysta*—Massuti Alzamora, *Notas Resúm. Inst. esp. Oceanogr.*, ser. II, **43**:19 (incorrect subsequent spelling).
- 1930 *Dyctiocusta*—Massuti Alzamora, *Notas Resúm. Inst. esp. Oceanogr.*, ser. II, **43**:20 (incorrect subsequent spelling).
- 1939 *Dictyocysta* Ehrenberg emended Kofoid and Campbell—Kofoid and Campbell, *Bull. Mus. Comp. Zool. Harv.*, **84**:284 (revision).
- 1968 *Dictyocysta* Ehrenberg, 1854—Loeblich and Tappan, *J. Protozool.*, **15**:187.
- 1979 *Dictyocysta* Ehrenberg, 1854—Corliss, *The Ciliated Protozoa*, 2nd ed.:306.
- 1994 *Dictyocysta* Ehrenberg, 1854—Laval-Peuto, *Traité de Zoologie*, II (2):208.
- 2001 *Dictyocysta* Ehrenberg 1854—Aescht, *Denisia*, **1**:59.
- 2008 *Dictyocysta* Ehrenberg, 1854—Lynn, *The Ciliated Protozoa*, 3rd ed.:351.

Improved diagnosis—Lorica firm, cyathiform, divided in a hyaline collar and a usually agglomerated bowl by a generally indistinct constriction. Collar cylindroidal, with one or two rows of windows larger than mullions, usually shorter than bowl. Bowl subspherical or obovoidal, often fenestrated. With lorica sac and foldable closing apparatus.

Type species—*Dictyocysta elegans* Ehrenberg, 1854 (by monotypy).

Discussion—Ehrenberg established the genus *Dictyocysta* twice, naming *D. elegans* in February (Ehrenberg 1854a) and *D. acuminata* and *D. lepida* in May (Ehrenberg 1854b). In the type species, Biedermann (1893) observed both a closing apparatus and a lorica sac. The “hyaline panes”, “translucent membranes”, or “sheets” closing the bowl windows in several species are probably part of the lorica sacs.

According to Kofoid and Campbell (1929, 1939) and Laval-Peuto (1994), the family Dictyocystidae is characterized by the possession of a closing apparatus. Besides *Dictyocysta*, the family includes the genus *Luminella* (Kofoid and Campbell 1939) or the genus *Wangiella* (Table 1; Lynn 2008). However, a closing apparatus is unknown in *Luminella* Kofoid & Campbell, 1939, which comprises *Stenosemella*-like species with collar windows. Furthermore, *Luminella* was recently synonymized with *Stenosemella* Jørgensen, 1924, as the type species of the latter genus was also found to possess collar windows (Agatha and Tsai 2008). In the type species of *Wangiella* Nie, 1934, the inner collar with its 16–18 prismatic flaps might correspond to a closing apparatus, while a lorica sac was not mentioned (Nie 1934); the fungus genus *Wangiella* established by McGinnis (1977) represents a junior homonym.

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Fig. 1–3.

Codonella aspera, schematic drawings of the closing apparatus combined from live observations and scanning electron micrographs. **1.** Lateral view of a shut closing apparatus showing five triangular folds in one half. The apparatus apparently originates from the lorica constriction (arrow), as the lorica sac lining the bowl is not visible. **2.** Top view of a shut closing apparatus, whose 10 slightly vaulted triangular valves are radially arranged, leaving a minute opening in the centre (arrow). **3.** Inner wall of an open closing apparatus. The shallow furrows (arrows) form the triangular folds in the closed apparatus (cf. Fig. 1). Together with the longitudinal ribs (arrowheads), they are responsible for an ordered folding of the apparatus. The ring-shaped crease (double arrowhead) at the base of the closing apparatus is attached to the lorica constriction in the live specimen. B, lorica bowl; C, lorica collar; CA, closing apparatus. Scale bars=20 μm (Fig. 1, 2) and 10 μm (Fig. 3).

Fig. 4–11.

Codonella aspera from life (4–6) and in the scanning electron microscope (7–11). **4, 5.**

Longitudinal optical sections of specimens with almost (4) and completely (5) shut closing

apparatuses (arrows) apparently originating from the lorica constrictions. **6.** Top view showing 10 radially arranged triangular valves (asterisks) in the shut closing apparatus. **7, 8.**

Top view and detail of a contracted specimen with a widely open closing apparatus. The shallow furrows (arrowheads), which form the triangular folds in the closed apparatus, are separated by valves with six to eight longitudinal ribs on the inner wall. **9, 10.** Top view (10)

and detail (9) of a half-closed apparatus. The arrowheads (9) denote the conspicuous triangular folds, while the arrow (10) marks the ring-shaped crease, which was attached to the inner wall of the lorica constriction in the live specimen. **11.** Oblique top view. The triangular valves leave a minute opening (arrowhead) in the centre of the shut closing apparatus. B, lorica bowl; C, lorica collar; CM, collar membranelles. Scale bars=20 μm

(Fig. 4–7, 10), 10 μm (Fig. 11), and 5 μm (Fig. 8, 9).

Fig. 12–18.

Codonella aspera in the scanning electron microscope. **12, 13.** Top view and detail of an almost shut closing apparatus showing the radial arrangement of 10 triangular folds and valves. Probably, the ring-shaped crease (12, arrow) was attached to the lorica constriction in the live specimen. **14.** Detail of a widely open closing apparatus. The longitudinal rib (arrow) on the outer wall of the membrane corresponds to a shallow furrow on the inner wall and becomes the top of a fold in the shut apparatus. **15.** Detail of a half-closed apparatus. The faint longitudinal stripes (arrowheads) probably correspond to the ribs on the inner wall of the closing apparatus (cf. Fig. 3, 7, 8); note that one stripe is proximally bifurcated. **16.** Lateral view of a lorica showing the constriction (arrow) between the bowl and the collar. **17.** Margin of a widely open closing apparatus, whose membrane is possibly composed of two layers (arrowhead). **18.** Top view of a half-closed apparatus. B, lorica bowl; C, lorica collar; CM, collar membranellae. Scale bars=40 μm (Fig. 16), 20 μm (Fig. 12, 18), 10 μm (Fig. 13, 15), and 5 μm (Fig. 14, 17).

Fig. 19–23.

Dictyocysta mitra in the scanning electron microscope (19–22) and *Dictyocysta lepida* from life (23). **19.** Lateral view of an extended specimen. Due to the reticulated lorica wall, it is obvious that the longitudinally wrinkled closing apparatus merges posteriorly into a lorica sac. An inconspicuous lorica constriction (arrow) separates the bowl and the collar. **20.** Oblique top view of a disturbed tintinnid with a widely open closing apparatus. Since contacts between the cell and the closing apparatus are not recognizable, there is no evidence for an active closure by the ciliate. **21, 22.** Lateral views of anterior tintinnid portions. The membranes of the lorica sacs are smooth or irregularly wrinkled, while the closing apparatuses (arrows; cf. Fig. 36, 37) are longitudinally striped and folded. The rather stiff ring-shaped crease (22, arrowhead) marks the base of the slightly sagged closing apparatus. **23.** Lateral view of a specimen with an almost shut closing apparatus, which forms conspicuous triangular folds (arrowhead) and apparently originates from the inner wall of the lorica constriction (arrow) separating bowl and collar. B, lorica bowl; C, lorica collar; CA, closing apparatus; CM, collar membranelles. Scale bars=40 μm (Fig. 19), 20 μm (Fig. 20, 23), and 10 μm (Fig. 21, 22).

Fig. 24–47.

Foldable (24–38) and diaphragm-like (39–47) closing apparatuses (arrows; 24–27, 33–37, from life; 29, after haemalum staining; 30–32, after application of trichloracetic acid; 28, 38, 45–47, preserved; 39–44, possibly unpreserved; 24, from Fol 1883; 25, from Daday 1887; 26, 27, 39–44, from Balech 1959; 28, original; 29, 37, from Entz 1909; 30–32, 34, 35, from Hofker 1931; 33, from Entz 1885; 36, from Biedermann 1893; 38, from Brandt 1906; 45–47, from Kofoed and Campbell 1929). **24–28. *Codonella aspera*.** Lateral views (24, 25), longitudinal optical sections (26, 28), and top view (27), 86 μm , 94 μm , 93 μm long, 48 μm across, 82 μm long. **29–32. *Codonaria cistellula*.** Longitudinal optical sections (29, 31), lateral view (30), and oblique top view (32), 87 μm long, ? μm , ? μm , ? μm . **33–35. *Dictyocysta lepida*.** Lateral views (33, 34) and longitudinal optical section (35), 75 μm long, ? μm , ? μm . **36, 37. *Dictyocysta mitra*.** Lateral views, 61 μm , 63 μm long. Kofoed and Campbell (1939) mentioned about four folds in one half of the closing apparatus, which corresponds to Fig. 37. **38. *Codonellopsis pacifica*.** Lateral view, 228 μm long. **39, 40. *Salpingella decurtata*.** Lateral views, 160 μm , 160 μm long. **41–43. *Salpingella rotundata*.** Lateral views, 134 μm , 138 μm , 141 μm long. **44. *Salpingella curta*.** Lateral view, 86 μm long. **45. *Salpingacantha perca*.** Lateral view, 190 μm long. **46. *Salpingacantha unguiculata*.** Lateral view, 155 μm long. **47. *Salpingacantha exilis*.** Lateral view, 218 μm long. LS, lorica sac separated from lorica bowl due to shrinkage caused by preservative. ?, unknown.

Table 1

Classification of extant tintinnid ciliates following Lynn (2008). Genera with closing apparatuses are in bold.

Family Ascampbelliellidae Corliss, 1960
Family Codonellidae Kent, 1881
Genus <i>Codonaria</i> Kofoid & Campbell, 1939
Genus <i>Codonella</i> Haeckel, 1873
Genus <i>Codonopsis</i> Kofoid & Campbell, 1939
Genus <i>Poroecus</i> Cleve, 1902
Genus <i>Tintinnopsis</i> Stein, 1867
Family Codonellopsidae Kofoid & Campbell, 1929
Genus <i>Codonellopsis</i> Jörgensen, 1924
Genus <i>Laackmanniella</i> Kofoid & Campbell, 1929
Genus <i>Stenosemella</i> Jörgensen, 1924 (syn. <i>Luminella</i> Kofoid & Campbell, 1939; see text)
Family Cyttarocylididae Kofoid & Campbell, 1929
Family Dictyocystidae Haeckel, 1873
Genus <i>Dictyocysta</i> Ehrenberg, 1854
Genus <i>Wangiella</i> Nie, 1934
Family Epiplocylididae Kofoid & Campbell, 1939
Family Metacylididae Kofoid & Campbell, 1929
Family Nolaclusiliidae Sniezek, Capriulo, Small & Russo, 1991
Family Petalotrichidae Kofoid & Campbell, 1929
Family Ptychocylididae Kofoid & Campbell, 1929
Family Rhabdonellidae Kofoid & Campbell, 1929
Family Tintinnidae Claparède & Lachmann, 1858
Genus <i>Albatrossiella</i> Kofoid & Campbell, 1929
Genus <i>Amphorellopsis</i> Kofoid & Campbell, 1929
Genus <i>Amphorides</i> Strand, 1928
Genus <i>Brandtiella</i> Kofoid & Campbell, 1929
Genus <i>Bursaopsis</i> Kofoid & Campbell, 1929
Genus <i>Buschiella</i> Corliss, 1960
Genus <i>Canthariella</i> Kofoid & Campbell, 1929
Genus <i>Clevea</i> Balech, 1948
Genus <i>Dadayiella</i> Kofoid & Campbell, 1929
Genus <i>Epicranella</i> Kofoid & Campbell, 1929
Genus <i>Eutintinnus</i> Kofoid & Campbell, 1939
Genus <i>Odontophorella</i> Kofoid & Campbell, 1929
Genus <i>Ormosella</i> Kofoid & Campbell, 1929
Genus <i>Proamphorella</i> Kofoid & Campbell, 1939
Genus <i>Prosteliella</i> Kofoid & Campbell, 1939
Genus <i>Rhabdosella</i> Kofoid & Campbell, 1929
Genus <i>Salpingacantha</i> Kofoid & Campbell, 1929
Genus <i>Salpingella</i> Jörgensen, 1924

Genus *Salpingelloides* Campbell, 1942

Genus *Steenstrupiella* Kofoid & Campbell, 1929

Genus *Stelidiella* Kofoid & Campbell, 1929

Genus *Tintinnus* Schrank, 1803

Family Tintinnidiidae Kofoid & Campbell, 1929

Family Undellidae Kofoid & Campbell, 1929

Family Xystonellidae Kofoid & Campbell, 1929

Table 2

Tintinnid species studied during the sampling periods in Villefranche-sur-mer. Species with closing apparatuses are in bold.

<i>Amphorides quadrilineata</i> (Claparède & Lachmann, 1858) Strand, 1928
<i>Climacocylis scalaria</i> (Brandt, 1906) Jörgensen, 1924
<i>Codonella aspera</i> Kofoid & Campbell, 1929
<i>Codonellopsis schabi</i> Brandt, 1906
<i>Dadayiella ganymedes</i> (Entz, 1884) Kofoid & Campbell, 1929
<i>Dictyocysta lepida</i> Ehrenberg, 1854
<i>Dictyocysta mitra</i> Haeckel, 1873
<i>Eutintinnus</i> spp.
<i>Favella</i> sp.
<i>Proplectella</i> sp.
<i>Rhabdonella brandti</i> Kofoid & Campbell, 1929
<i>Rhabdonella spiralis</i> (Fol, 1881) Brandt, 1907
<i>Salpingella acuminata</i> (Claparède & Lachmann, 1858) Jörgensen, 1924
<i>Steenstrupiella steenstrupii</i> (Claparède & Lachmann, 1858) Kofoid & Campbell, 1929
<i>Stenosemella ventricosa</i> (Claparède & Lachmann, 1858) Jörgensen, 1924
<i>Tintinnidium</i> spp.
<i>Tintinnopsis</i> spp.
<i>Xystonella longicauda</i> Brandt, 1906

Table 3

Characteristics of the closing apparatus in *Codonella aspera*.

Characteristics	$\bar{X}$	M	SD	SE	CV	Min	Max	n
Shut closing apparatus, height ^a	9.7	9.8	1.8	0.4	18.3	7.2	13.0	18
Folds/valves, number ^b	10.2	10.0	–	–	–	10.0	11.0	9
Folds, height ^b	5.7	5.8	0.4	0.2	7.2	5.0	6.1	7
Folds, length ^b	19.7	19.0	3.1	0.9	15.7	17.0	25.0	11
Membrane, thickness ^c	0.1	0.1	–	–	–	0.1	0.2	5

^aFrom live specimens. Distance between loricula constriction and top of the folds.

^bFrom scanning electron micrographs.

^cFrom scanning electron micrographs; only rough values as membrane margin was rarely seen in top view and difficult to measure.

CV, coefficient of variation in %; M, median; Max, maximum; Min, minimum; n, number of individuals investigated; SD, standard deviation; SE, standard error of arithmetic mean; $\bar{X}$, arithmetic mean.